SIMULTANEOUS ACID AND ALKALINE BACTERIAL FERMENTATIONS FROM DEXTROSE AND THE SALTS OF ORGANIC ACIDS RESPECTIVELY

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I. AN EXPLANATION OF REVERSION OF REACTION OF CULTURE MEDIUMS BY ORGANISMS OF THE COLON-AEROGENES GROUP

INTRODUCTION

Acid and alkaline bacterial fermentations, as they are usually conceived, involve the splitting up of carbohydrates or similar carbon-containing substances and protein or its decomposition products, respectively. There is no doubt that acids are produced from sugars and undoubtedly ammonia is formed by many bacteria as an end product of protein decomposition; nevertheless an alkaline reaction is not necessarily due to ammonia or alkaline basic decomposition products from protein.

It is strange that the many investigators who have noted alkaline fermentations did not attempt to explain these fermentations on the basis of something other than ammonia, which their figures often showed was not present in amounts sufficiently large to account for the total alkalinity. In some cases the alkaline reaction has been accounted for by either ammonia or alkaline basic substances from protein decomposition perhaps in an attempt to make up the discrepancy between the ammonia actually found and the alkaline reaction.

The fact that the salts of many organic acids may be converted into alkaline carbonates through bacterial agencies has long been recognized but the significance of that type of fermentation seems to have been overlooked. As early as 1878 Hoppe-Seyler[1] found that calcium tartrate and citrate with putrid fibrin gave calcium carbonate as one of the end products of the fermentation. A little later he found that calcium bicarbonate and hydrogen resulted from the fermentation of calcium formate and mud. Maassen[2] worked with 52 varieties

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[1] Ztschr. Physiol. Chem., 1879, 2, p. 1.
[2] Arb. a. d. k. Gsndhtsamte, 1895, 12, p. 340.

of bacteria and the sodium salts of 21 organic acids and found that carbonates were formed from the organic salts. It was found by Pakes and Jollyman,[3] who studied the bacterial decomposition of formic acid, that sodium formate could be split up by B. coli into sodium bicarbonate and hydrogen. The same decomposition was also noted by Harden.[4]

From the studies with the same type of organism, Grey[5] believes that calcium formate may split into calcium carbonate, carbon dioxid and hydrogen. Emmerling[6] also states that calcium carbonate could be formed by the bacterial fermentation of calcium tartrate. It has been shown by Omelianski[7] that potassium carbonate is formed by the fermentation of potassium acetate and that calcium carbonate resulted from the fermentation of calcium butyrate. Other organic acid salts besides those already mentioned can be converted into carbonates as is shown by the work of Gingingham.[8] He isolated from soil bacteria which could convert calcium lactate into calcium carbonate when driven to it by lack of other food. Evidently bacteria which are capable of converting the salts of organic acids into carbonates are common in soil, for Temple[9] found that a solution of 1 gm. of potassium dibasic phosphate in 1,000 c.c. of water containing sodium citrate, potassium citrate or sodium potassium tartrate turned alkaline on incubation when previously inoculated with soil. He made the following statement:

"It is clear that soil bacteria can use the sodium or potassium salts of organic acids in such a way as to have one of the products sodium or potassium carbonate."

No attempt will be made to cite all the references in the literature to the bacterial fermentations of salts of organic acids, as there have been many pieces of work reviewed in which other organic acids were formed during the fermentation of a given organic acid salt.

Our attention has been focused on the alkaline fermentation of organic acid salts through an extensive study of the alkaline-forming group of bacteria. In a preliminary paper[10] we called attention to the fact that the alkaline reaction in milk produced by the alkaline-forming group of bacteria was due to the fermentation of salts of citric acid and a production of alkaline carbonates. It was also pointed out that that group of bacteria could ferment the sodium salts of numerous organic acids and convert them into alkaline carbonates. During some later work on the alkali-forming bacteria it was found that some of them could ferment dextrose and it therefore seemed probable that two fermentations, one acid (fermentation of sugar), and the other

[3] Jour. Chem. Soc. (Lond.), 1801, 79, p. 386.
[4] Ibid., p. 610.
[5] Proc. Roy. Soc. Lond., 1914, B, 87, p. 461.
[6] Centralbl. f. Bakteriol., 1908, II 21, p. 317.
[7] Ibid., 15, p. 673.
[8] Jour. Agr. Sci., 1911, 4, p. 145.
[9] Ga. 'Agr. Exper. Stat., 1914, Bul. 103.
[10] Ayers, S. H., and Rupp, Philip: Science, 1915, 42, p. 318.

alkaline (fermentation of organic acid salts) could progress simultaneously.

Knowing that the organisms of the colon type could utilize carbon from organic acid salts and cause an alkaline reaction it seemed evident that the reversion of reaction in culture mediums by organisms of the colon-aerogenes group as described by Clark and Lubs[11] could be explained by a simultaneous acid and alkaline fermentation. In their first work on the differentiation of the colon-aerogenes group by means of indicators Clark and Lubs[11] used a medium containing 0.5% potassium dibasic phosphate, 0.5% dextrose, 0.5% peptone and distilled water. They found that after five days' incubation at 30 C. cultures of B. coli which gave a low gas ratio $\frac{Co_2}{H}=\frac{1}{1}$ showed a high hydrogen-ion concentration and gave a red color with methyl-red. On the other hand, cultures of B. aerogenes which gave a high gas ratio $\frac{Co_2}{H}=\frac{2}{1}$ showed a low hydrogen-ion concentration and were yellow to methyl-red. It was pointed out by Clark and Lubs that when the sugar content and "regulator" is propertly adjusted the colon type soon reach a high limiting hydrogen-ion concentration which remains constant while the aerogenes type exhausts the sugar before the limiting hydrogen-ion concentration is reached, a reversion in reaction takes place and the medium becomes more alkaline.

It may be assumed off hand that the reversion in reaction is due to the production of ammonia through decomposition of the peptone, although Clark and Lubs pointed out that it should not be assumed that the reversion is due solely to an ammonia production.

In our first experiments it was found that the reversion took place in a synthetic medium in which no ammonia could be liberated from the decomposition of peptone.

THE REVERSION IN A MEDIUM CONTAINING SODIUM-AMMONIUM-PHOSPHATE AS A SOURCE OF NITROGEN

Believing that the reversion by members of the colon-aerogenes group was not due to ammonia resulting from the decomposition of peptone a synthetic medium was prepared. Sodium-ammonium phosphate was used as a source of nitrogen, as that salt had been extensively used in work with the alkali-forming group of bacteria. The composition of the medium was as follows: sodium-ammonium-phosphate 3.6 gm., potassium-acid-phosphate 1.2 gm., dextrose 5 gm., and 1,000 c c distilled water.

[11] Jour. Infect. Dis., 1915, 17, p. 160.

Throughout these experiments we have used a low gas ratio culture termed Fg and a high ratio culture termed Ze. These cultures were supplied to us through the kindness of Mr. Rogers of this laboratory. For convenience Culture Fg is considered B. coli and the Culture Ze, B. aerogenes.

When grown in the very simple sodium-ammonium-phosphate medium it was found that a reversion of reaction occurred with Culture Ze and not with Fg. The hydrogen-ion change in each culture is seen in Figure 1. The B. coli Culture Fg went progressively acid reaching the region of P_H 4.8 while the B. aerogenes Culture Ze reached P_H 5.5 then went progressively alkaline so that on the 6th day it had reached P_H 6.3. These cultures could be differentiated on the 2nd day by methyl-red as Fg had reached P_H 4.9 while Ze was in the region of P_H 5.9.

It was evident from these results that it was possible to get a reversion in a medium free from peptone, which strengthened our opinion that the reversion in a peptone medium was not due to the production of ammonia. The reversion in the sodium-ammonium-phosphate medium could be due only to the formation of bicarbonates or carbonates from the salts of the organic acids produced through the fermentation of the dextrose. Any ammonia liberated from the sodium-ammonium-phosphate through bacterial action would either be taken up immediately by the acid produced in the fermentation of the sugar, in which case it would form an ammonium salt of the acid which would be converted into bicarbonate or carbonate, or it would combine with the acid phosphate to form again sodium or potassium-ammonium-phosphate.

While this work was in progress Clark and Lubs[12] published a paper in which they showed that a reversion took place in a medium containing acid-ammonium-phosphate as a source of nitrogen. They lowered the content of total nitrogen to a point at which its participation in any form in changes of reaction of the medium would become insignificant and showed that an extensive reversion of reaction took place under such conditions. They therefore concluded that in such cases it would be impossible to attribute the reversion to the liberation of ammonia.

The reversion of the reaction is obviously not due to the production of ammonia and as will be shown later is the result of a simultaneous acid and alkaline fermentation in which the salts of the organic acids produced from the sugar are converted into bicarbonates or carbonates.

SIMULTANEOUS ACID AND ALKALI FERMENTATIONS IN A SODIUM-AMMONIUM-PHOSPHATE MEDIUM

The composition of sodium-ammonium-phosphate medium has been given in this paper. Two series, of 6 flasks each, of this medium were prepared and 6 of the flasks were inoculated with a B. coli Culture Fg and the other 6 flasks

[12] Jour. Biol. Chem., 1917, 30, p. 209.

with an B. aerogenes Culture Ze. Each flask contained 990 c c of medium and was inoculated by adding 10 c c of a fresh culture in the same medium which had been incubated at 30 C. for 24 hours. The flasks were then incubated at 30 C. and one flask containing Fg and one containing Ze were examined for sugar, volatile acids, and its hydrogen-ion concentration after 1, 2, 3, 4, 8, and 14 days' incubation.

The dextrose was determined as cuprous oxid by the official method of Munson and Walker.[13] The quantity of formic acid in the medium was found by experiment not to interfere with the determination of dextrose.

The method for the determination of the volatile acids was as follows: 800 c c of the medium were distilled with phosphoric acid until 700 c c had passed over. The residue was then distilled with steam in a 500 c c flask until a 100 c c of the distillate required less than 0.5 c c of N/10 caustic soda solution for neutralization. The total distillate was evaporated to dryness, dissolved in 100 c c distilled water, filtered, and diluted to 200 c c. The formic acid was determined by oxidation with mercuric chlorid according to the method of Franzen and Eggers[14] and the acetic acid was found by difference.

TABLE 1

FERMENTATION OF DEXTROSE IN A SODIUM-AMMONIUM-PHOSPHATE MEDIUM

Days	Culture—Fg (Low Ratio)					Culture—Ze (High Ratio)				
	P_H	Grams of Dextrose Fermented	C C of N/10 Volatile Acid	Grams of Formic Acid	Grams of Acetic Acid	P_H	Grams of Dextrose Fermented	C C of N/10 Volatile Acid	Grams of Formic Acid	Grams of Acetic Acid
1	5.6	0.9340	114.55	.3245	.2644	6.0	1.8380	103.98	.3098	.2201
2	5.0	1.3080	118.50	.3249	.2876	5.8	3.4980	104.71	.3289	.1998
3	5.0	1.3500	120.88	.3285	.2973	5.7	4.5920	95.90	.3079	.1715
4	4.9	1.4480	118.65	.3194	.2959	6.1	4.6200	67.50	.1911	.1560
8	4.8	1.5280	125.75	.3285	.3265	6.5	4.6420	46.98	.0429	.2261
14	4.8	1.5040	127.13	.3421	.3170	6.5	4.6520	71.03	.0046	.4204
Control Flask 1,000 C C	6.9	Dextrose Grams 4.8240	2.79	.0067	.0080	6.9	Dextrose Grams 4.8080	2.62	.0063	.0073

If, as was suspected, a simultaneous acid and alkaline fermentation took place, then as the fermentation progressed the organic acids should not increase in a constant proportion to the amount of dextrose fermented. An examination of Table 1 shows that such was the case. With Culture Fg (B. coli) the reaction reached P_H 4.8 on the 8th day and remained constant. It will be noted, however, that the volatile acid did not increase in proportion to the amount of sugar fermented, although it followed much more closely than in the case of Culture Ze (B. aerogenes).

In the colon series the amount of formic acid remained fairly constant while the acetic acid increased slightly. With the aerogenes

[13] U. S. Dept. Agr., Bur. Chem. Bul., 1908, 107.

[14] Jour. Prakt. Chem., 1911, 83, p. 323.

culture the hydrogen-ion concentration increased to P_H 5.7 on the 3rd day, then a reversion in reaction took place and on the 8th day the reaction had reached its lowest point, P_H 6.5. While with Fg approximately 1.5 gm. of sugar were used out of a total of about 4.8 gm., Culture Ze utilized about 3.5 gm. within 48 hours and at the end of 72 hours about 4.6 out of a total of about 4.8 gm. were fermented. With this rapid fermentation of sugar it is seen in the table that the amount of total volatile acid decreased after 48 hours' incubation. In

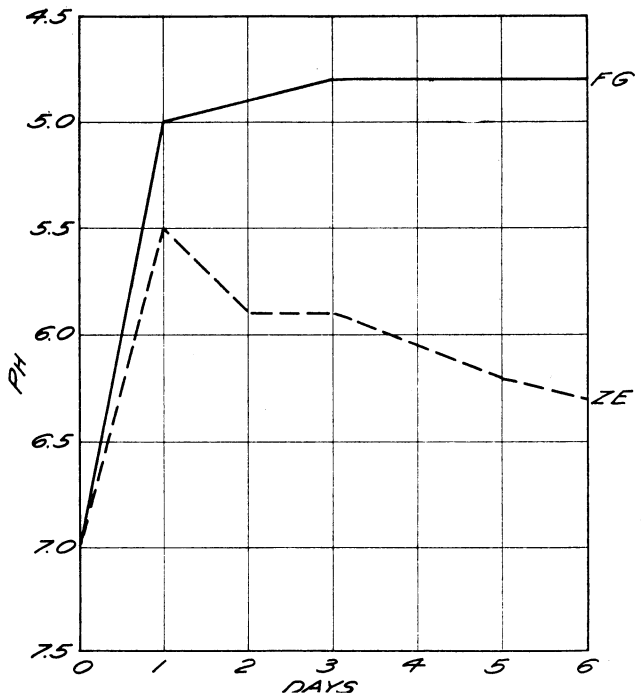

Fig. 1.—The reversion of reaction in a sodium-ammonium-phosphate-dextrose medium by organisms of the colon-aerogenes group.

fact about twice as much dextrose was used after the 2nd day incubation as after 1 day and yet the total volatile acid increased only from 103.98 to 104.71 c c. It is of further interest to observe that the amount of formic acid began to decrease on the 3rd day and there was only a trace left on the 14th day. The acetic acid decreased slightly at first but increased markedly between the 8th and 14th days. This will be discussed later.

The simultaneous fermentation of the sugar and the salts of the organic volatile acids is best shown in Figure 2. In the graph the amount of sugar fermented is plotted on a different scale from the amount of volatile acid but the curves can be compared. It is evident that if the volatile acid was not utilized by the bacteria then it would bear a definite ratio to the amount of sugar fermented. In the case of the B. coli Culture Fg it may be seen from the curves that this is true to a large extent. During the first 48 hours, however, there was

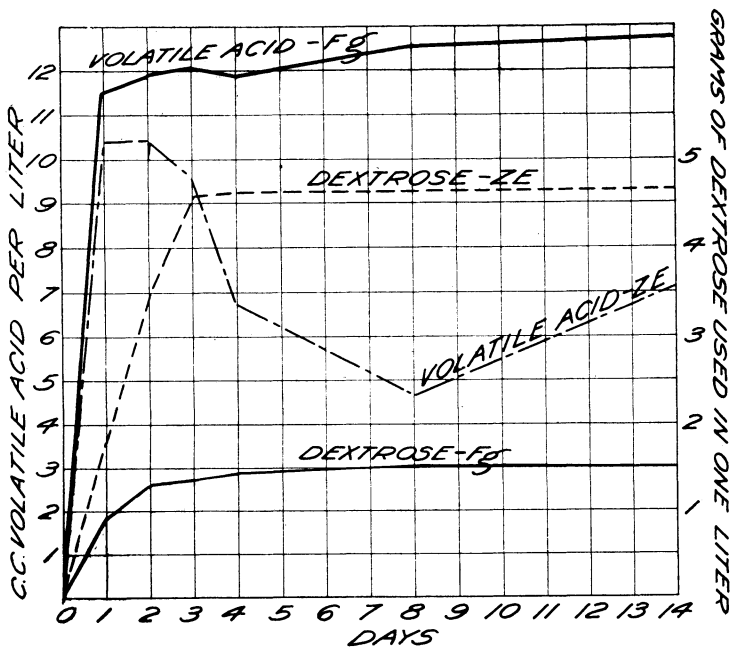

Fig. 2.—Dextrose fermented and amount of volatile acid produced by cultures Fg and Ze.

considerable difference in the ratio, showing that some of the volatile acids was used with the sugar.

B. coli in a sodium-ammonium-phosphate medium soon reaches its highest hydrogen-ion concentration with the fermentation of a relatively small amount of sugar and when this high acid reaction is reached the sugar fermentation practically ceases. Until the highest hydrogen-ion concentration is reached there is evidently a simultaneous acid and an alkaline fermentation which must tend to neutralize each other.

Further examination of Figure 2 reveals the fact that the simultaneous fermentation is more strikingly in evidence in fermentations of B. aerogenes Culture Ze. There was a rapid fermentation of the sugar and also the salts of the volatile acids. The curve shows that the total volatile acid on the 2nd day is about the same as on the 1st, and that there is a rapid decrease followed by an increase after the 8th day. In aerogenes fermentations there is a rapid destruction of the dextrose and at the same time a rapid fermentation of the volatile acids resulting from the sugar fermentation. The salts of the organic acids are oxidized to carbonates or bicarbonates which tend to neu-

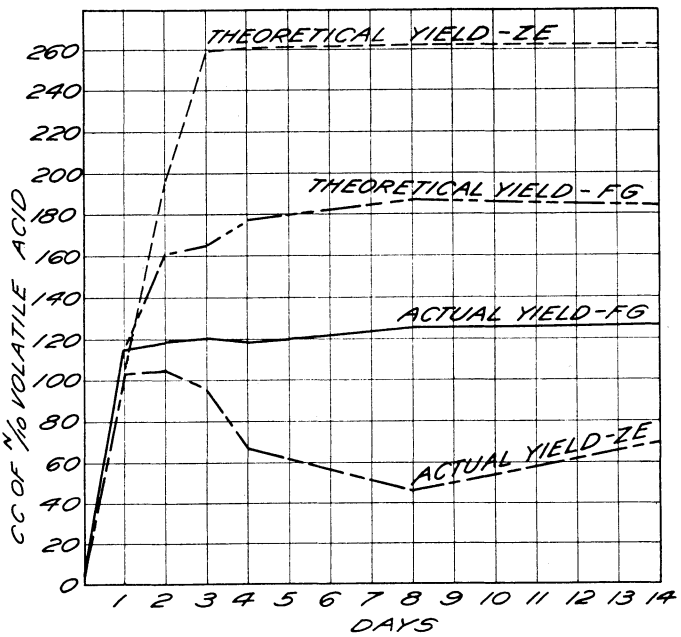

Fig. 3.—Theoretical amount of volatile acid compared with the amount formed.

tralize the acidity, therefore retarding the rise to the highest hydrogen-ion concentration, provided the relation of sugar to buffer is favorable. When the sugar is almost completely used, and it is apparent that it is not entirely used, the alkaline fermentation of the salts of the volatile acids progresses at an increased rate. The increased rate may be due to the partial removal of one source of carbon, the sugar, leaving only the salts of the organic acids or it may be due to the increased amount of the salts of the organic acids in the medium at the time the sugar

is nearly exhausted, or it may be due to both conditions. Since there is no further production of acids the effect of the alkaline fermentation is to cause a reduction in acidity and a reversion of reaction.

Further proof of simultaneous fermentation of sugar and salts of volatile acids is presented in Figure 3. Curves have been plotted to show the amount of volatile acids determined and the theoretical amount which should have been found based on the amount of sugar fermented. As a basis for calculation the proportion of volatile acid present to sugar fermented at the end of 24 hours' incubation was used. It is plain from the graph that much less volatile acid was found than should have been present based on the amount of dextrose fermented. B. aerogenes Culture Ze, it is noted, ferments the salts of the volatile organic acid much more vigorously than the B. coli Culture Fg.

Unfortunately in the determination of the organic acids, the solutions set away and containing the lactic and succinic acids resulting from the bacterial fermentation, molded so that determination of those acids could not be made. It was therefore necessary to repeat the experiment using the same medium in order to determine the formic, acetic, lactic, and succinic acids resulting from the fermentation of the dextrose.

The method of determining lactic and succinic acids was as follows: The residue from the steam distillation of the volatile acids was filtered, neutralized with caustic soda, and evaporated to about 90 c c. This liquid was then acidified with phosphoric acid and extracted with ether for 14 hours in a continuous extracting apparatus. The residue from the ether extract was heated with 100 c c of water and pulverized calcium carbonate on a steam bath for 2 hours, shaking occasionally. The solution was then filtered through an aluminum crucible, washed with hot water, and diluted to 200 c c. In 50 c c of the filtrate the calcium, corresponding to the lactic plus the succinic acids, was determined as oxalate. The remaining 150 c c were evaporated to dryness and the residue taken up with 10 c c of hot water. When cool 90 c c of absolute alcohol were added, the mixture allowed to stand for 2 hours, shaking occasionally, then filtered and washed with 90% alcohol. The filtrate was evaporated to remove the alcohol and calcium of the lactate determined as oxalate. The succinic acid was found by difference.

If, as in the former experiment, the results were plotted and compared, the P_H values, the dextrose fermented and the formic and the acetic found would be practically identical. This fact served as an excellent check on the experiment and showed that fermentation under controlled conditions progressed at a very definite rate.

The results, however, are expressed in a different manner in order to bring out some new points and to emphasize further the simultaneous acid and alkaline fermentation. The results in Table 2 show the

amount of dextrose fermented and the per cent. of formic, acetic, lactic and succinic acid based on the amount of sugar fermented after 1, 4, 8, and 14 days' incubation at 30 C. as well as the hydrogen-ion concentration of the medium.

The B. coli Culture Fg used up about 1.5 gm. of dextrose and in 14 days reached a P_H value of 4.8. It is noted that the percentage of formic acid decreased slightly during the first 4 days and then remained nearly constant. There was practically no change in the percentage of acetic, lactic, or succinic acid during the fermentation. It is of interest to observe that although the highest hydrogen-ion concentration of P_H 4.8 was reached on the 4th day the fermentation did not stop although the rate was slower. This is shown by the increase in the

TABLE 2

ORGANIC ACIDS PRODUCED BY FERMENTATION OF DEXTROSE IN A SODIUM-AMMONIUM-PHOSPHATE MEDIUM

Culture	Days	P_H	Grams of Dextrose Fermented	Per Cent. of Acids Based on Amount of Sugar Fermented			
				Formic	Acetic	Lactic*	Succinic
Fg (low ratio)	1	5.3	1.2440	29.17	23.21	Lost	Lost
	4	4.8	1.4680	23.32	22.27	16.92	4.66
	8	4.8	1.5080	22.42	22.36	15.25	5.09
	14	4.8	1.5120	22.71	22.65	15.17	5.38
Ze (high ratio)	1	5.9	2.3100	13.29	11.44	2.58	1.68
	4	6.0	4.8120	5.14	3.05	2.28	1.53
	8	6.5	4.8440	1.72	4.19	1.75	1.38
	14	6.5	4.8480	0.21	8.60	0.90	0.45
Control Flask 1,000 C C		6.9	Dextrose 5.0080	Grams of Formic Acid .0076	Grams of Acetic Acid .0139	Grams of Lactic and Succinic Acids .0202	

amount of dextrose used from the 4th to the 14th day. The results with the B. aerogenes Culture Ze were quite different. This organism rapidly fermented nearly all the sugar and used up nearly all the formic acid. During the first 4 days the acetic acid was also fermented as is shown by the lowered per cent., but it is also seen that the acid increased between the 4th and 14th day of incubation. This increase of acetic acid may be due to the oxidation of alcohol formed during the fermentation or to the action of the Culture Ze on lactic and succinic acid. Our results do not indicate that lactic and succinic acids are used to any great extent by Culture Ze although the results show some decrease in the per cent. It is interesting, however, to observe that only small amounts of those acids were produced in the fermentation by Ze as compared to the amount produced by Fg, which is also

true of the volatile acids formic and acetic. It seems probable that the salts of the organic acids are fermented almost as rapidly as produced, thereby making it impossible to determine accurately the amount formed during the sugar fermentation.

The acids produced by the fermentation of dextrose in a synthetic medium using a colon and aergones organism can be seen best in Figures 4 and 5, in which the results previously discussed are plotted. After observing the curve for lactic acid and comparing its position with those of formic and acetic acid one is led to wonder at the statement often found in textbooks that the colon bacillus forms lactic acid from sugar with small amounts of volatile acids. This evidently is

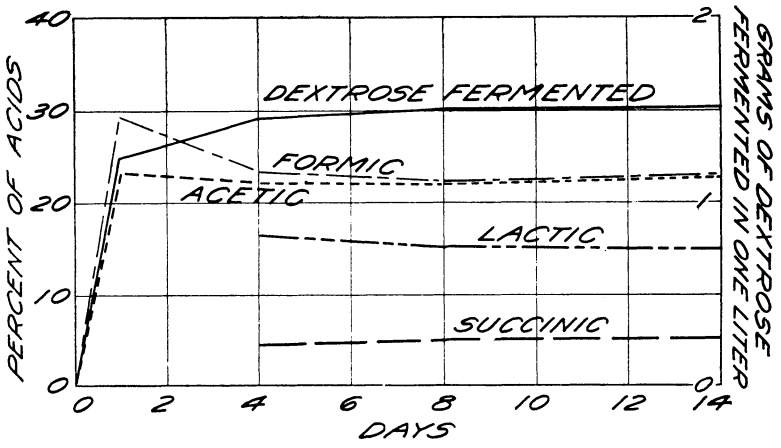

Fig. 4.—Per cent. of various organic acids formed from dextrose by a coli culture.

not true for the fermentation of dextrose in a synthetic medium such as was used in our experiment. The end products of a sugar fermentation, however, vary in accordance with the composition of the medium and the period of incubation.

THE EFFECT ON REVERSION OF REACTION OF THE ADDITION OF SODIUM SALTS OF ORGANIC ACIDS TO THE CULTURE MEDIUM

To this point we have shown that when dextrose is fermented in a medium containing sodium-ammonium-phosphate as a source of nitrogen, the simultaneous fermentation may take place with the colon organism to a slight extent while it is actively produced by the aerogenes type. In this fermentation the dextrose is split into organic

acids which are undoubtedly at once converted into bicarbonates or carbonates.

If this belief is correct then the addition of sodium salts of the acids formed during the fermentation of dextrose to a medium containing dextrose should hasten the reversion of the reaction. We should expect this to assist particularly the aerogenes culture which as has been shown acts most vigorously on the salts of the organic acids.

To determine this effect of the addition of salts of the organic acids 3 mediums were used with the following composition:

No. 1

Sodium-ammonium-phosphate	3.6 gm.
Potassium-acid-phosphate	1.2 gm.
Dextrose	5.0 gm.
Distilled water	1000 c c.

No. 2

Same medium as No. 1, plus 1.2 gm. of the organic acid to be studied. This acid was neutralized with sodium hydrate.

No. 3

Sodium-ammonium-phosphate	3.6 gm.
Potassium-acid-phosphate	1.2 gm.

1.2 gm. of organic acid neutralized with sodium hydrate.

This gave 3 mediums, one with dextrose as a source of carbon, one with both dextrose and the salt of an organic acid, and one with only the salt of the organic acid to supply carbon.

The effect of sodium formate was tried first, as previous results showed that that acid was fermented most readily by the aerogenes culture. Five tubes of each medium were inoculated with the B. coli Culture Fg and the same number with B. aerogenes Culture Ze. One tube of each series was examined for the hydrogen-ion concentration after 1, 2, 3, 5, and 6 days of incubation at 30 C.

It is seen from Figure 6 that both cultures ferment sodium formate and caused a progressive alkaline change when there was no dextrose in the medium. This alkaline change must be due to either bicarbonate or carbonate. The curve of the hydrogen-ion concentration in the dextrose formate medium with coli (Fg) falls below that of the plain dextrose medium. A determination of the increased buffer effect due to the addition of sodium formate showed that it would account for the difference in the curves after the 2nd day of incubation. The difference in hydrogen-ion concentration during the first 48 hours, however, can not be explained by the increased buffer and signifies a

decrease in the hydrogen-ion concentration due to a slight fermentation of the sodium formate.

The effect of the presence of sodium formate on a dextrose fermentation by aerogenes (Ze) is strikingly shown by the curves. When only dextrose was present Ze reached a maximum of P_H 5.2 while in the dextrose-formate medium the maximum was 6.0. Throughout the fermentation the hydrogen-ion concentration of the medium containing dextrose and formate was distinctly lower than that with only dex-

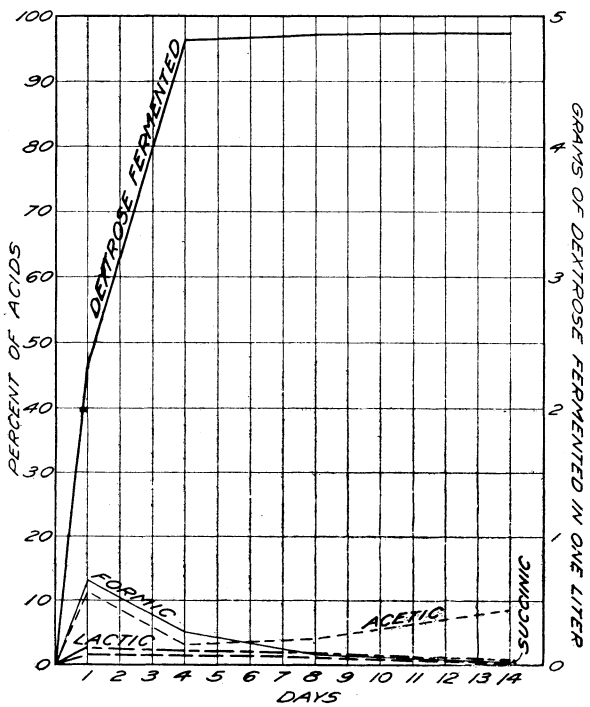

Fig. 5.—Per cent. of organic acids formed from dextrose by an aerogenes culture

trose. The increased buffer effect of the medium due to sodium formate was found to account for only a small part in the lowering of the hydrogen-ion concentration.

When sodium acetate was substituted for sodium formate the effect was much the same as that of the formate. Figure 7 shows, however, that when sodium acetate was the only source of carbon the reaction of the medium did not become so alkaline as with the formate. While the addition of sodium acetate lowered the hydrogen-ion concen-

tration throughout the fermentation its effect was not so marked as that of sodium formate. Here again the increase in the buffer by the addition of sodium acetate plays a part in the lowering of the hydrogen-ion concentration, but it accounts for only a small part of the lowered hydrogen-ion concentration.

The fact that the salts of formic and acetic acid assist in the reversion of reaction by B. aerogenes when added to a dextrose medium together with figures previously presented showing that those acids are

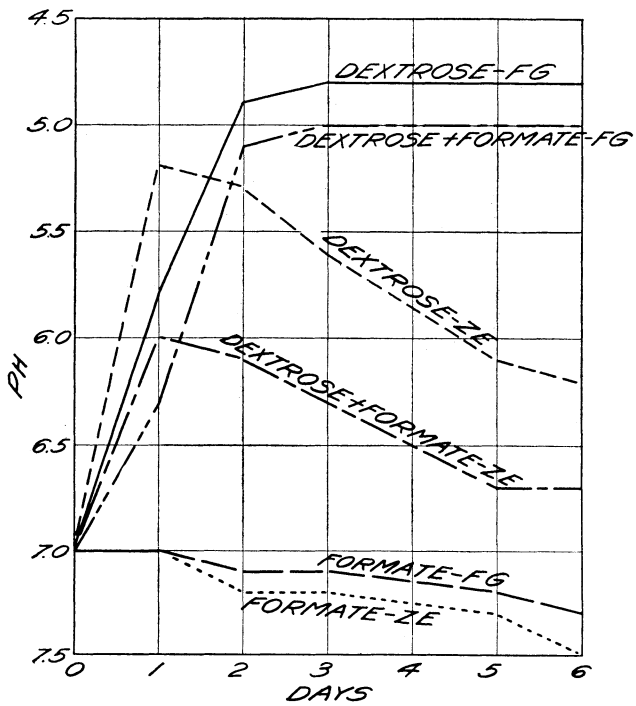

Fig. 6.—Influence of sodium formate on the reversion of reaction by members of the colon-aerogenes group.

used in a dextrose medium in which they were formed from the sugar, seems positive proof that the reversion in reaction with aerogenes cultures is due to their oxidation to alkaline carbonates. We believe that the fermentation of the formate plays the most important part in the reversion since, as has already been shown, it is used more rapidly than the salts of the other acids. The fermentation of the lactate and the succinate do not appear to play any important part although it is

possible that the salts of all the organic acids produced in the fermentation may influence the reversion in reaction as caused by the aerogenes culture.

SIMULTANEOUS ACID AND ALKALINE FERMENTATIONS IN A PEPTONE MEDIUM

From the preceding results it is evident that the reversion in reaction brought about by the aerogenes type of organisms in the synthetic medium used was not due to the production of ammonia. It may be

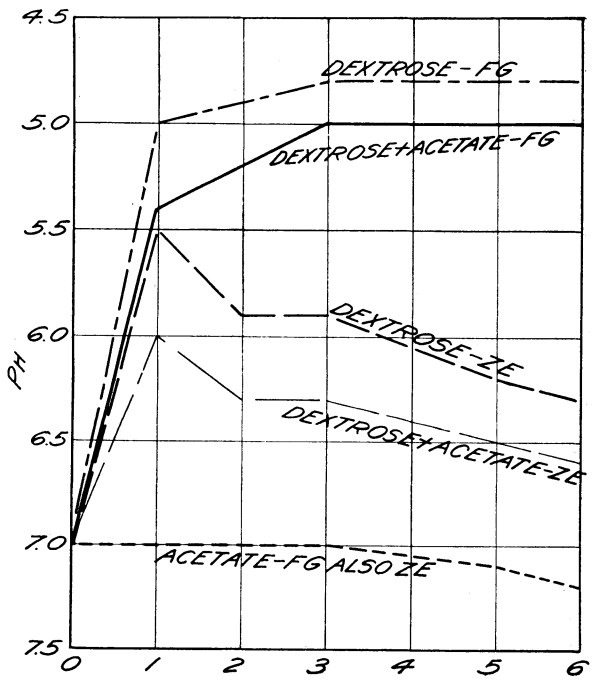

Fig. 7.—Influence of sodium acetate on the reversion of reaction by members of the colon-aerogenes group.

argued, however, that the reversion in a synthetic medium when no free ammonia can be liberated is due to the alkaline fermentation of the salts of the organic acids, but that in a peptone medium the cause of the reversion is the liberation of ammonia as the end-product of protein decomposition. To the bacteriologists the mention of an alkaline reaction in a medium brings to the mind the thoughts of the decomposition of nitrogenous bodies. Thus it is perhaps only natural

that the reversion of reaction in a peptone medium should be assumed to be due to the liberation of ammonia.

We believed, however, that the reversion in a peptone medium was due to an alkaline fermentation of the salts of the organic acids the same as in the synthetic medium and not to ammonia. In order to determine if this were true a study was made of the fermentation in a peptone medium as used by Clark and Lubs.[11]

The medium was made as they directed and comprised the following named ingredients:

Witte's peptone	0.5%
Potassium-acid-phosphate	0.5%
Dextrose	0.5%
Distilled water	

Two series of flasks each of this medium were prepared and one series was inoculated with the B. coli Culture Fg and the other with the B. aerogenes Culture Ze. Each flask contained 990 c c of medium and was inoculated by

TABLE 3

FERMENTATION OF DEXTROSE IN A DEXTROSE-PEPTONE MEDIUM

Culture	Days	P_H	Grams of Dextrose Fermented	Per Cent. of Acids Based on Amount of Sugar Fermented		Milligrams of Ammonia per 100 C C Medium
				Formic	Acetic	
Fg (low ratio)	1	5.1	1.9400	21.69	22.32	4.32
	2	5.0	2.3000	16.60	19.85	4.54
	3	4.9	2.5240	13.25	18.94	2.61
	5	4.9	3.2800	0.12	16.03	2.81
	14	4.9	3.5080	trace	16.14	4.54
Ze (high ratio)	1	5.7	3.8240	12.81	4.81	2.44
	2	5.5	4.9880	9.48	3.27	3.06
	3	6.2	4.9560	7.49	3.23	1.48
	5	6.3	4.9500	0.97	4.74	2.04
	14	6.3	4.9360	trace	12.87	5.12
Control Flask 1,000 C C		6.9	Dextrose 5.0560	Grams of Formic Acid .0029	Grams of Acetic Acid .0171	4.54

adding 10 c c of a culture in the same medium which had been incubated at 30 C. for 24 hours. The flasks were then incubated at 30 C. and one flask of each series was examined for sugar, volatile acids, ammonia, and the hydrogen-ion concentration of the medium after 1, 2, 3, 5, and 14 days. The ammonia was determined by Folin's method and the sugar and volatile acids by methods previously described.

Let us consider first the fermentation by the B. coli Culture Fg. The most interesting feature of this fermentation in a peptone medium is that it varies somewhat from that in a synthetic medium. A comparison of the results in Tables 2 and 3 shows that more than

twice as much sugar was fermented by Fg in a peptone medium as when the source of nitrogen was sodium-ammonium-phosphate. In the peptone medium nearly all the formic acid was used up in 5 days, while in a synthetic medium it was used only to a slight extent. It is of further interest to note that the ammonia was never found in quantities greater than the amounts in the control flasks and so evidently played no part in the reaction.

We wish to call particular attention to the fact that as shown in Table 3 the P_H value reached 4.9 on the 3d day in the colon fermentation and then remained constant in spite of the fact that the amount of dextrose fermented increase from about 2.5 gm. to about 3.5 gm. between the 3d and 14th day of incubation. Evidently this organism does not cease its activities in a peptone-dextrose medium after a certain zone of hydrogen-ion concentration is reached, as believed by Clark and Lubs.[11] It would seem rather that a particular zone of hydrogen-ion concentration is reached and at that point the rate of acid and alkaline fermentations becomes so balanced as to keep the reaction constant, thus allowing further fermentation of the sugar and salts of the organic acids, particularly formic. According to the table the formic acid was used up very largely between the 3d and 14th day of incubation while the reaction remained constant.

In the fermentation of the B. aerogenes Culture Ze there was little difference in the peptone and sodium-phosphate medium, as may be noted by a comparison of the results in Tables 2 and 3. The principal point of interest in this fermentation is the fact that the hydrogen-ion concentration reaches its highest point, 5.5, on the 2nd day and then the medium went progressively alkaline until P_H 6.3 was reached. Keeping that fact in mind the amount of ammonia present in the medium should be observed. Since, with the exception of slight increase between the 5th and the 14th day, there was less ammonia than in the control, it seems evident that the reversion of reaction was not due to ammonia. It should be noted, however, that the formic acid was used up the same as in the synthetic medium.

The prevailing idea has been that an alkaline reaction was evidence of the decomposition of nitrogenous matter with the production of ammonia. This misconception seems to have firmly planted itself in the minds of most bacteriologists who have attempted to explain the reversion of reaction in the coli-aerogenes group. Thus Kligler[15] states: "The progressive change produced in carbohydrate media by

[15] Jour. Bacteriol., 1916, 1, p. 663.

members of the colon typhoid group, excepting certain types, is from alkaline to acid and back. So long as there remains unutilized carbohydrates the acid phase persists. With the complete consumption of the carbohydrates the organism actively attacks the nitrogenous components of the medium, neutralizing the acid and gradually returning to the alkaline phase."

Levine[16] also considers that the alkali is produced from peptone for he says: "It may further be considered that after the limiting hydrogen-ion concentration is reached the organism, if not destroyed, will if capable attack peptones forming alkali. Some of the free acid becomes neutralized and more carbohydrate may be decomposed."

Again we find a similar opinion expressed in the statement of Burton and Rettger[17] writing on the correlations of the colon-aerogenes group: "Following the loss of all the sugar there will occur more or less protein decomposition depending on its availability and thus a certain amount of ammonia production will take place which would account for the alkalinity of some cultures."

So far as our experiments are concerned it is evident that the reversion in reaction by at least one aerogenes culture is due to a simultaneous fermentation of carbohydrate and the organic acid salts produced in its fermentation. The difference between the coli and aerogenes culture is one of rate, the final hydrogen-ion concentration being the result of the rates of the acid and alkaline fermentations. The aerogenes culture ferments the sugar more rapidly than the coli and as the sugar becomes exhausted the rate of fermentation of the organic acid salt becomes greater and the resulting alkaline carbonates cause the reversion in reaction.

It is not strange that the attempts to explain the reversion of reaction in the coli-aerogenes group have been based on the formation of ammonia from protein after the exhaustion of the sugar. Moreover, it is only natural in view of the extensive work by Kendall and his associates on the sparing action of carbohydrates on protein decomposition. In the future, however, the importance of alkaline fermentations of the salts of the organic acids must receive careful consideration.

CONCLUSION

In conclusion we wish to review the facts previously discussed and to offer an explanation of the reversion of reaction in culture mediums by organisms of the colon-aerogenes group.

[16] Jour. Infect. Dis., 1916, 19, p. 773.
[17] Ibid., 1914, 14, p. 411.

It should be understood thoroughly that our results apply to a reversion in reaction in the synthetic medium used in our work and in the peptone medium used by Clark and Lubs.[11] We know that when small amounts of sugar are present and the buffer action of a peptone medium properly adjusted, ammonia may be formed which can produce a reversion in reaction. We believe, however, that in general the reversion in reaction is due to the fermentation of organic acid salts rather than to the formation of ammonia.

The reversion of reaction was produced by an aerogenes culture in a synthetic medium in which ammonia could not cause the reversion.

In a synthetic medium a simultaneous acid and alkaline fermentation took place. While organic acids were produced from the fermentation of the dextrose the salts of those acids were fermented at the same time with the sugar. Sodium formate was found to be utilized to the greatest extent and sodium acetate to a less extent. The salts of lactic and succinic acids were only slightly fermented. Since sodium formate was extensively fermented it seems evident that the alkaline change was due largely to its oxidation to bicarbonates or carbonates.

The addition of sodium salts of formic and acetic acids to a synthetic medium containing dextrose hastened the reversion by an aerogenes organism. Sodium salts of lactic and succinic acids had little or no effect on the reversion of the reaction by the same organism.

In a dextrose-peptone medium as used by Clark and Lubs the reversion in reaction was accompanied with the same simultaneous fermentation of dextrose and salts of the organic acids, particularly the formate. Ammonia played no part in the reversion since there was less ammonia found than in the control uninoculated medium, while the reaction changed from P_H 6.9 to P_H 5.5 and back to P_H 6.3 during a period of 5 days.

Having reviewed briefly the facts brought out in this paper let us now consider the probable explanation of the reversion in reaction by members of the colon-aerogenes group.

1. Dextrose is fermented by both colon and aerogenes organisms and organic acids are formed, which acids combine with the dibasic phosphate-forming salts of the organic acids and acid phosphates, the latter giving an acid reaction.

2. As soon as organic acid salts are formed they are at once fermented simultaneously with the dextrose. Since formic-acid salts are fermented to the greatest extent it is evident that bicarbonates or car-

bonates must be formed; therefore the acid and alkaline fermentations progress simultaneously.

3. It is probable that bicarbonates cause the reversion in reaction, since the formic acid salt is utilized to a much greater extent than the salts of the other organic acids. If the carbonates are formed they would probably be converted into bicarbonates in the presence of the carbon dioxid in the medium.

4. As bicarbonates are produced they react with the acid phosphate in the medium liberating carbon dioxid and forming dibasic phosphate which reacts alkaline and causes a reversion of the reaction. The essential process and the one on which the other adjustments of equilibrium depend is the replacement of a relatively strong organic acid by a relatively weak carbonic acid. Furthermore at the P_H values observed Co_2 must be lost causing an increase in the P_H. For example, equal parts of formic acid and sodium formate in solution have a P_H value of 3.7. When completely oxidized to equal parts of carbonic acid and sodium bicarbonate the P_H value is increased to 6.5. The carbonates lose Co_2 slowly and there is a further increase in the P_H.

5. The amount of formate fermented by an aerogenes culture during the reversion of reaction practically accounts for the change in hydrogen-ion concentration is shown by the following figures. Reference to Table 1 shows that on the 3rd day in the fermentation of Ze the sugar was practically exhausted and the hydrogen-ion concentration had reached its highest point P_H 5.7. At the same time the amount of formic acid present was 0.3079 gm. per liter. On the 8th day a reversion in reaction had taken place and the P_H value was 6.5 while only 0.0429 gm. of formic acid was found. To change the reaction from P_H 5.7 to 6.5 in the medium used 64 c c of N/10 alkali would be required. Calculating the amount of N/10 bicarbonate resulting from the fermentation of the formate during the reversion of the reaction it was found that 0.2650 gm. of formic acid, the amount fermented between the 3rd and the 8th day, corresponded to 57.6 c c of N/10 bicarbonate. Since only 64 c c were required to change the reaction from P_H 5.7 to 6.5 it is evident that the fermentation of the formic acid, in the form of its salts, practically accounted for the reversion in reaction. The alkali fermentation of other organic acid salts probably accounts for the small amount of alkaline change not accounted for by the fermentation of the formate.

6. Colon and aerogenes cultures both produce a simultaneous fermentation of the dextrose and the salts of the organic acids under proper conditions and the reason the aerogenes cultures revert is because they ferment the dextrose and organic acid salts at a different rate from those of the colon cultures.

II. SIMILAR FERMENTATIONS BY ORGANISMS OF THE ALKALI-FORMING GROUP OF BACTERIA

INTRODUCTION

As has been stated, our attention was drawn to the possibility of simultaneous acid and alkaline fermentations, from sugar and the salts of organic acids, respectively, through a study of the alkali-forming group of bacteria. This group comprises those bacteria which produce an alkaline reaction in milk under aerobic conditions without causing visible signs of peptonization. This alkaline reaction is not the result of ammonia formation but, as we[10] have previously reported, is due to the formation of alkaline carbonates resulting from the oxidation of citric acid in milk.

It is quite apparent that the ability of an organism to carry on two types of fermentation at the same time, one acid and the other alkaline from two different sources of carbon, greatly complicates the interpretation of sugar fermentations. These simultaneous fermentations, as has been shown, play an important part in the fermentations of the colon-aerogenes group but, as we shall see, they are not confined to that group.

SIMULTANEOUS ACID AND ALKALINE FERMENTATIONS AMONG THE ALKALI-FORMING GROUP OF BACTERIA

In order to show that the alkali-forming bacteria utilize two sources of carbon simultaneously, 3 different cultures were studied and each was grown in 3 different mediums. In one medium the only source of carbon was dextrose, in another sodium citrate, and in the third the carbon was supplied by both dextrose and sodium citrate. Sodium citrate was used rather than other organic acid salts as the lactate, formate, etc., since these salts would be produced in the fermentation of the dextrose and so complicate the determination of the amount of organic acid salt fermented. Sodium-ammonium-phosphate was used as a source of nitrogen so as to provide a medium of definite composition.

The composition of the mediums was as follows:

No. 1	No. 2	No. 3
Dextrose .....5.0 gm.	Citric acid (neutralized with NaOH) .....2.5 gm.	.....2.5 gm.
Sodium-ammonium-phosphate .....1.5 gm.	.....5.0 gm.	.....1.5 gm.
Potassium chloride....0.2 gm.	.....1.5 gm.	.....0.2 gm.
Distilled water.....1,000 c.c.	.....0.2 gm.	.....1,000 c.c.
	.....1,000 c.c.	

Seven flasks of each medium were inoculated and one flask of each was examined after 1, 2, 3, 5, 7, 10, and 14 days of incubation at 30 C. Determinations were made of the hydrogen-ion concentration, the amount of dextrose, and sodium citrate fermented.

For the determination of citric acid, the following reagent was found superior to Deniges reagent which contains too large an excess of sulfuric acid:

Red oxid of mercury	50 gm.
Concentrated sulfuric acid	100 c c.
Water sufficient to make	1,000 c c.

To 50 c c of the medium in a 200 c c graduate flask was added 50 c c of the mercury reagent, the mixture diluted to 200 c c and filtered after 15 minutes. One hundred c c of the filtrate were heated to about 75 C. and a 1% solution of potassium permanganate added, drop by drop, until the precipitate acquired a light buff color, or the shade of a light manila envelope. At first the oxidation was slow but after the addition of 5 or 6 drops of permanganate solution it proceeded rapidly. The solution was heated to boiling, allowed to cool, filtered through a Gooch crucible, and the precipitate washed. It was then dried at 100 C. and weighed. The weight of precipitate multiplied by 0.2708 gave the amount of citric acid.

As may be expected when the course of a fermentation is traced by means of examinations of a number of individual flasks, there was found to be variations in the amount of carbon utilized from day to day. These variations, however, as may be seen in Table 4, were not sufficiently great to interfere seriously with the results. A study of the results reveals the fact that either sodium citrate or dextrose was fermented when present as the only source of carbon and that both were used when in the same medium.

The reactions produced by the fermentation of sodium citrate, dextrose, and both together, is best shown by the hydrogen-ion concentration plotted in Figure 8. Culture 10 fermented sodium citrate and caused a progressive alkaline change in the medium reaching P_H 8.5. With dextrose as a source of carbon an acid fermentation was produced, P_H 4.5 being reached on the 5th day. When both sodium citrate and dextrose were in the medium, it is noted that the acidity increased slightly on the 1st day, then the reaction went progressively alkaline.

When comparing the hydrogen-ion concentration of the dextrose and dextrose and citrate mediums during the course of the fermentation of these alkali-forming bacteria, we must not lose sight of the fact that the buffer actions of the two mediums are entirely different. The addition of sodium citrate greatly increases the buffer action of the medium and therefore lowers the hydrogen-ion concentration as the acid fermentation progresses. From this it is apparent that it is unsafe to assume offhand, that the difference in the hydrogen-ion concentration in the fermentation of dextrose alone and dextrose with

TABLE 4

FERMENTATION OF DEXTROSE AND SODIUM CITRATE BY ALKALI-FORMING BACTERIA

Culture No.	Days	Medium Containing Citrate		Medium Containing Dextrose		Medium Containing Citrate and Dextrose		
		PH	Milligrams of Citrate Fermented in 100 c c	PH	Milligrams of Dextrose Fermented in 100 c c	PH	Milligrams of Citrate Fermented in 100 c c	Milligrams of Dextrose Fermented in 100 c c
Control		7.6	253.3 in 100 c c	7.3	471.2 in 100 c c	7.6	249.3 in 100 c c	448.0 in 100 c c
10	1	7.6	15.1	6.8	43.1	7.3	0.0	2.0
	2	7.8	25.6	6.3	73.4	7.4	0.4	10.7
	3	7.8	24.6	4.6	89.9	7.4	11.0	21.5
	5	8.2	45.8	4.5	97.6	7.7	25.4	26.0
	7	8.2	46.5	4.5	91.6	7.8	42.3	32.0
	10	8.3	56.7	4.6	82.8	7.9	56.7	67.3
	14	8.5	57.7	4.5	94.4	8.0	91.5	67.3
21	1	7.6	0.9	7.0	34.4	7.4	2.3	4.7
	2	7.6	2.8	6.9	40.0	7.3	19.1	16.0
	3	7.7	8.1	6.8	52.4	7.4	35.3	18.0
	5	7.8	14.8	6.3	115.8	7.3	24.0	41.3
	7	7.9	29.1	6.0	78.2	7.4	40.1	48.0
	10	8.2	45.6	6.0	95.6	7.2	47.3	65.3
	14	8.2	42.7	5.4	91.2	7.1	56.3	64.0
110	1	7.6	0.0	6.8	64.4	7.2	0.0	62.6
	2	7.6	1.1	5.9	103.0	7.3	29.3	71.3
	3	7.7	13.2	4.5	108.6	7.4	9.7	18.7
	5	8.0	32.6	4.5	148.0	7.5	12.1	84.4
	7	8.2	48.6	4.5	122.2	7.5	31.8	30.0
	10	8.3	67.9	4.5	97.6	7.3	38.2	115.3
	14	8.5	71.6	4.5	112.2	6.6	68.1	158.0

sodium citrate is due to production of alkaline carbonates. It is easy to measure the buffer action of the sodium citrate and determine what part it plays in reducing the hydrogen-ion concentration if we assume that it is not used up during the fermentation. The buffer action of this salt is, however, complicated by the fact that in the fermentation it is constantly being fermented and its buffer effect consequently lost. Assuming, however, that it remains in a constant amount throughout the fermentation it was found that its buffer action was not anywhere near great enough to give account for the hydrogen-ion concentration determined during the fermentation of dextrose and sodium citrate.

Since the fermentations with Culture 10 did not show any variations throughout the series, we have plotted in Figure 9, the amount of sodium citrate and dextrose fermented and the reaction in terms of P_H values. The curve shows that after 1 day the acidity had increased slightly which correlates with the fact that at that time some dextrose had been fermented but no citrate. On the 2nd day the reaction had started on its alkaline course and some citrate had been fermented. It

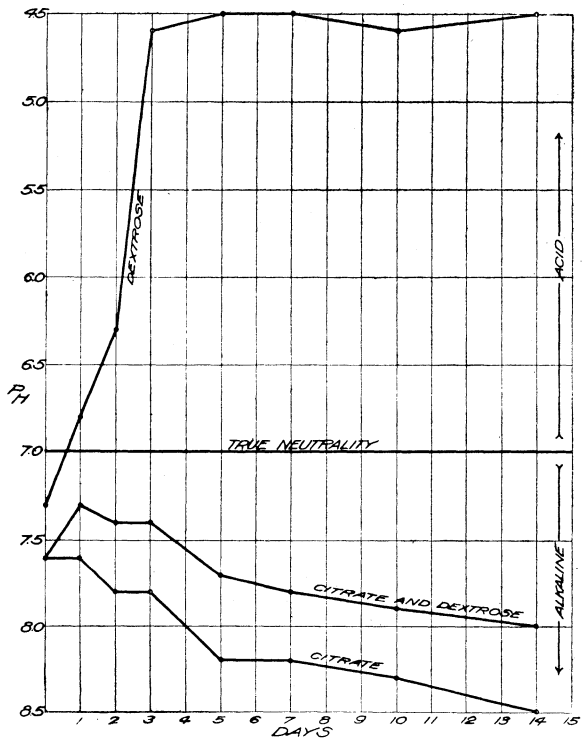

Fig. 8.—Reaction produced by fermentation of dextrose and sodium citrate, separate and together, by Culture 10.

was a small amount, however, and here the possibility must be noted that there may have been some alkaline carbonates formed from the fermentation of organic acid salts produced from the fermentation of the dextrose. This is a probability which exists throughout the experiment. This. however, does not detract from the results which show that the sodium citrate and dextrose were fermented to about the same

extent during the first 11 days while the reaction was constantly going alkaline.

The reaction resulting from the simultaneous acid and alkaline fermentation among the alkali-forming bacteria depends on the rate of fermentation of the sugar and the salts of the organic acids, and the buffer effect of the medium. As has just been seen with Culture 10 and 14 days' incubation 67.3 mg. of dextrose was fermented and

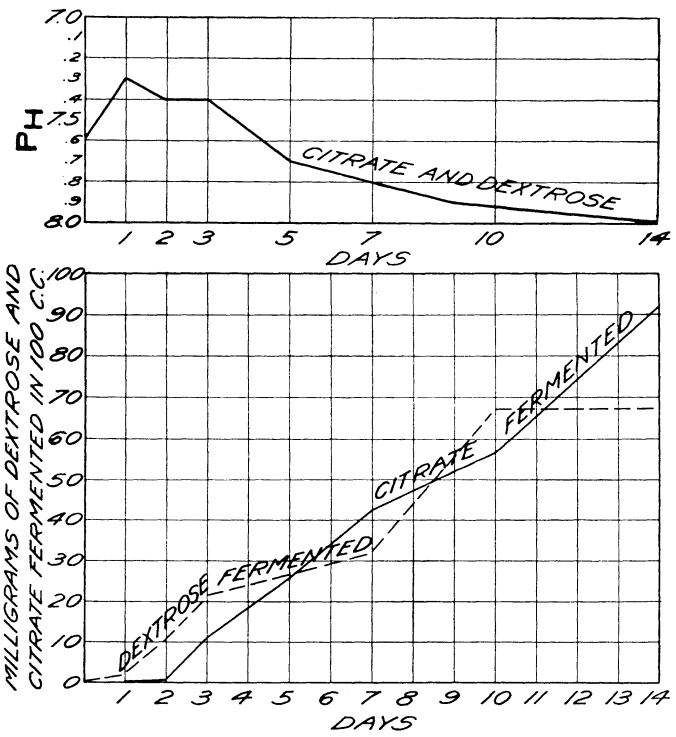

Fig. 9.—Amount of detrose and sodium citrate fermented in same medium by Culture 10 together with the hydrogen ion concentration of the medium.

91.5 mg. of sodium citrate, and as a result the reaction was changed from P_H 7.6 to P_H 8.0 an alkaline change.

Culture 31, on the other hand, fermented 64 mg. of dextrose and 56.3 mg. of sodium citrate when both were present in the same medium, and the hydrogen-ion concentration changed as is shown in Figure 10, from P_H 7.6 to P_H 7.1, a slight acid change. This culture caused an alkaline fermentation with sodium citrate when used as the

only source of carbon. In the dextrose fermentation the type of curve is quite different from that of Culture 10 for the acidity increased slowly and only reached P_H 5.4. In spite of the fact about the same amount of dextrose was fermented by both cultures as reference to Table 4 shows. This points to an active fermentation by Culture 31 of the salts of the organic acids produced in the fermentation of the dextrose.

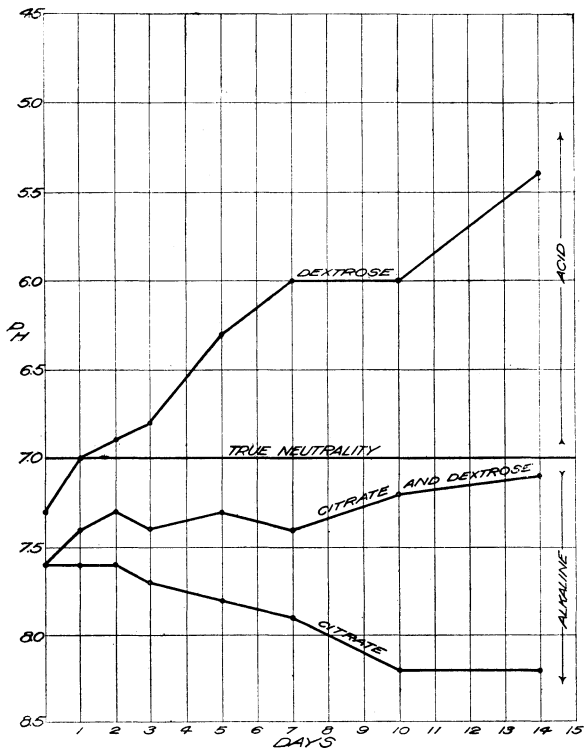

Fig. 10.—Reaction produced by fermentation of dextrose and sodium citrate, separate and together, by Culture 31.

Culture 110 showed even different results in the fermentation of sodium citrate and dextrose in the same medium. After 14 days 158.0 mg. of dextrose and only 68.1 mg. of citrate were fermented and as a result the reaction changed as is shown in Figure 11, from P_H 7.6 to P_H 6.6, a distinct acid change.

These results have been discussed more fully perhaps than would seem to be warranted at first thought. If, however, these simultaneous fermentations are clearly understood their far-reaching importance will at once be evident.

SIGNIFICANCE OF SIMULTANEOUS ACID AND ALKALINE FERMENTATIONS

Since an organism can ferment sugar and form organic acids and at the same time ferment the salts of the same acids and oxidize them to alkaline carbonates it is plain that these simultaneous fermentations may occur in any medium containing a fermentable sugar. Even in a synthetic medium this may occur if the organism is capable of utilizing

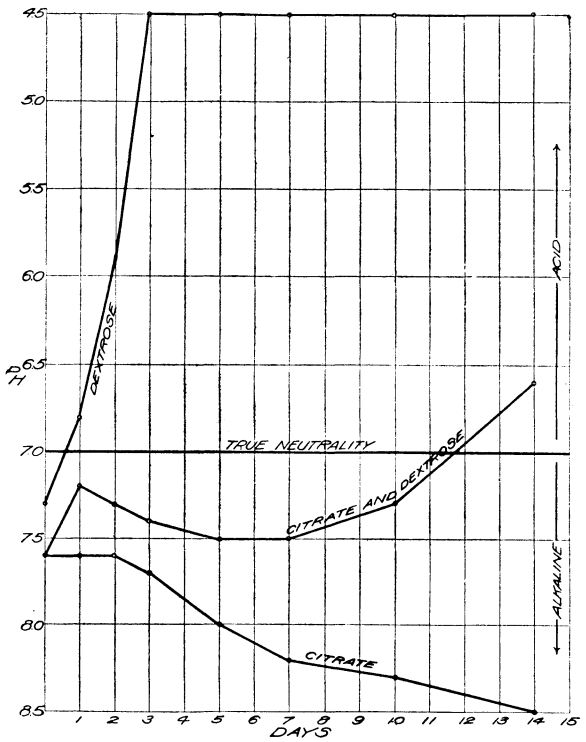

Fig. 11.—Reaction produced by fermentation of dextrose and sodium citrate, separate and together, by Culture 110.

carbon from the salts of the particular organic acids formed in the sugar fermentation. When the source of nitrogen in a medium is some definite substance like sodium-ammonium-phosphate, the hydrogen-ion concentration is a measure of the resultant of the acid and alkaline fermentation and under definite conditions of incubation, and when the sugar and buffer are properly adjusted, the reaction should be a measure of the rate of the two simultaneous fermentations devel-

oping from the primary fermentation of the sugar. In a synthetic medium free from organic acid salts the sugar must of course first be attacked, as there are no organic acid salts present. We may speak therefore, under these conditions of the primary sugar fermentation producing acid and the secondary organic acid salt fermentation producing alkaline carbonates. These terms "primary" and "secondary" are probably correctly used only in a theoretical consideration of the fermentation, since as soon as organic acid salts are produced from the sugar they can be immediately fermented, after which the two fermentations progress simultaneously.

The ordinary mediums contain organic acid salts, for they usually contain beef extract or meat infusion. This is especially true of meat infusions which are freed from sugar by fermentations with B. coli. In such cases not only are there organic acid salts normally present in the meat infusion but in addition those produced through the coli fermentation of the sugar. These sugar-free mediums, as well as sugar broths containing beef extract, have been and are now commonly used in the study of sugar fermentations by bacteria.

When sugar fermentations are conducted in such mediums the reaction may be due to the acid fermentation of the sugar and an alkaline fermentation not only of the salts of the organic acids produced from the sugar but those in the medium before inoculation. These simultaneous fermentations greatly complicate the significance of acid tests for purposes of classification, a fact which has been justly recognized. Thus Rogers, Clark and Davis[18] writing about alkali formation by organisms of the colon group, state: "This property of alkali formation with the subsequent tendency to uncontrolled variation, reduces very materially the value of the titer of sugar broths for diagnostic purposes." Also, Levine[16] after studying the acid production of coli-like bacteria concludes that acid formation should not be given precedence over gas formation in studies on B. coli, for the acid may be masked by a secondary alkali production.

Here we wish to call attention again to the fact that the acid and alkaline fermentations progress simultaneously so long as there is a sufficient quantity of sugar and organic acid salts to be fermented. The alkaline fermentation of the salts of the organic acids must not be considered as a secondary reaction in the sense that it occurs after the sugar is exhausted. This may at times be apparent since in some cases the revision in reaction comes after several days of incubation

[18] Jour. Infect. Dis., 1917, 21, p. 162.

and when it may be reasonably assumed that the sugar is nearly exhausted. In such cases the rate of the alkaline fermentation of the organic acid salts may be accelerated but it is not the beginning of the fermentation. The term "secondary fermentation" therefore should not be applied since it is liable to give a false conception of the true fermentations.

The fact that these simultaneous fermentations complicate and decrease the value of acid determinations in sugar fermentations in ordinary mediums should not discourage the use of sugar fermentations for diagnostic purposes. Rather should it lead to a broader conception of sugar fermentations and the application of the principles of the acid and alkaline fermentations of a sugar, if we may call it such.

As we have stated, under controlled conditions, knowing the ability of bacteria to obtain carbon from sugar and salts of organic acids which can be formed from the sugar, it should be possible to measure the rates of the two fermentations and put them to some practical diagnostic use." As an example, we may mention the "Methyl-red test" devised by Clark and Lubs[11] which operates on the principle of simultaneous fermentations, although they were not clearly understood when the test was made. Further studies of various groups of bacteria based on these fermentations should reveal characteristics of value for diagnostic purposes.

SUMMARY AND CONCLUSIONS

1. Simultaneous acid and alkaline fermentations may be produced by members of the alkali-forming group of bacteria through the fermentation of sugar and salts of organic acids.

2. In a synthetic medium containing dextrose and sodium citrate an acid and alkaline fermentation takes place and the dextrose and citrate are used simultaneously.

3. The rate of fermentation of the dextrose and citrate varies with different cultures.

4. Simultaneous acid and alkaline fermentations, where carbonates are formed from the oxidation of salts of organic acids, may complicate and detract from the value of the determination of acidity in sugar fermentation. Under controlled conditions, however, the simultaneous fermentations can probably be used to considerable advantage for diagnostic purposes.